**Part 5 – Metabolism**

Energy production: fermentation, an/aerobic respiration, chemolitho- and phototrophy.

**Def. metabolism**: The sum of all biochemical reactions ⬄ catabolism + anabolism  
**Def. catabolism**: Degradation of complex molecules into less complex ones. Energy is made available.  
**Def. anabolism**: Production of complex carbohydrates from less complex ones. Energy is used.

Microorganisms perform chemotrophy or phototrophy. As C-sources, they can use CO2 or sugars and other reduced carbons.

**Redox reactions**

Redox reactions save energy in ATP. The electron donor passes its electrons to the accepter and is thus oxidized while the other is reduced. Redox reactions consist of two half reactions, the reducind and the oxidizing reaction. The reduction potential describes the willingness of a chemical species to give away its electrons. The more negative the reducing part the stronger the reduction.  
Equation: deltaG\_0 = -nFdeltaE\_0, deltaE\_0 should be positive for the reaction to happen spontaneously.

NAD+ is an electron transporter and is not consumed in redox reactions. In exergonic reactions, ATP is used and 32 kJ/mol are released. In endergonic reactions, ATP is produced, therefore, energy is needed.

**Substrates and energy**

**High energy substrates and their bonds**: ATP (anhydrid and ester bond), PEP (anhydrid bond), Acetyl CoA (thioester bond), G6P (ester bond), acetylphosphat (anhydrid bond).

There are two ways for ATP to be won: substrate level phosphorylation and electron transport coupled phosphorylation. On the substrate level, ATP can be won from PEP (pyruvate kinase), acetyl phosphate (acetate kinase) and 1,3-biphospho glycerate (phosphoglycerokinase). In the CAC, succinyl CoA to succinate is another substrate level source of ATP production.

Two ATPs are won during 2 PEP 🡪 2 pyruvate and 2 1,3-BPG 🡪 2 3-PG.

**Fermentation**: Anaerobic glycolsis which takes place when there is no electron acceptor present. Pyruvate is fermented to lactose with lactose dehydrogenase.

**Lactic acid bacteria**: Lactic acid bacteria perform fermentation, they are anaerobic but aerotolerant, they are grampositive Stäbchen and Kokken, they do not need iron and need some vitamins (they are auxotroph).

Homofermentative microbes only produce lactate through glycolysis, while heterofermentative microbes produce CO2, ethanol, acetate and lactate without glycolysis (use other pathways). For heterofermentative microbes, pentoses are more attractive as a substrate since two ATPs can be won from pentoses.

Peroxidases catalyze H2O2 + NADH + H+ 🡪 water + oxygen + NAD+.

**Different types of fermentation**

In ethanol fermentation, pyruvate is decarboxylated to acetaldehyd, losing CO2 via pyruvate decarboxylase. Acetaldehyd 🡪 ethanol, enzyme is alcohol DH and NAD+ is generated.

In mixed fermentation, there is ethanol and lactate fermentation as well as a third path. Third path: pyruvate 🡪 2 formiate + acetyl CoA, enzyme: pyruvate formiat lyase. Acetyl-CoA 🡪 Acetyl-P, enzyme: phosphotransacetylase, which is made into acetate, generating ATP. Formiate 🡪 H2 + CO2 via formiate hydrogen lyase.

**Enterobacteria and H2**: pyruvate + CoA-SH 🡪 acetyl-CoA + formiate via pyruvate formiate lyase. Then, formiate + H+ 🡪 H2 + CO2 via formiate hydrogen lyase.

**Archaea, anaerobic grampositive bacteria and anaerobic eukaryotes and H2 as their fermentation product**: pyruvate + 2Fd\_ox + CoA-SH 🡪 acetyl-CoA + 2Fd\_red + CO2 + H+ via pyruvate ferrodoxin oxidoreductase. Then, 2Fd\_red + 2H+ 🡪 H2 + 2Fd\_ox.

**Further fermentation pathways**: Butter acid and butanol/acetone fermentation in Clostridium spp., amino acid fermentation (Stickland reaction), propion acid fermentaion (lactate is further metabolized in propion bacteria).

**Def. syntrophy**: A process, where two or more organisms work together to degrade a substance, which none of them could do alone. Examples: secondary fermentation, species-wide hydrogen transfer, steps in axonic C-cycle.

**Electron transport chain**

Features: membrane associated, transfer of primary electron donor to terminal acceptor, some freed energy is conserved, ATP synthesis through ATP synthase, involved are NADH DH, flavoproteins, cytochroms, iron-sulphur proteins, creation of pH gradient and proton motive force for ATP production, NADH is electron donor, electron carriers in the membrane are sorted with increasing positive reduction potential, final carrier transfers it to terminal acceptor, e.g. O2 (becomes H2O).

NAD(P)+/NAD(P)H reduction potential = -0.32 V. Oxygen is + 0.82 V. deltaE\_0 = +1.14 V. deltaG = 216 kJ/mol for transfer of NADH to O2.

Some **redox systems** can transfer both electrons and protons: Flavins and quinones. Others can only transfer electrons such as cytochroms and Fe-S proteins.

**Complexes in electron transport chain in aerboic respiration**: complex 1 = NADH DH, complex 3 = ubiquinol-cytochrome-c-oxidoreductase, complex 4 = cytochrome-c-oxidase.

10H+ are pumped over the membrane in aerobic respiration.The ATP synthase is necessary for the production of ATP and also its reverse reaction, the hydrolysis of ATP. It is made of F1 and F0 subunit. F1 subunit can hydrolyze ATP on its own, while for synthesis both subunits are needed. F0 is a proton channel, F1 is a multiprotein complex that extends into the cytoplasm.

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**Methanogens**

Methanogens are anaerobic bacteria and archaea that use methane and CO2 as their main carbon source. Their substrates are CO2/H2 (hydrogenotroph), methylated substrates (methylotroph), and acetate (acetoclastic). Also, their electron donors are H2 (hydrogenic), H2S (sulphur), ammonia and nitrite, phosphite and iron (for chemolithotroph organisms). Chemolithotrophs metabolize NH3 and NO2- to NO3-. Many simply use CO2. Chemolithotrophs regenerate NADH with the reverse electron transport chain.

**Note**: Depending on how the C-atom is reduced in the substrate, one can gain more or less energy. For energy gain, it is: CO2 < glucose < fatty acids < methane (methane best, since it is more reduced than all other substrates).

The cow’s gut system contains bacteria that help digest cellulose and further ferment it to fatty acids such that fatty acids enter the cow’s blood stream and support it with energy. Also, the bacteria can be digested as a protein and amino acid source. The cow’s Pansen contain 10\*\*10 – 10\*\*11 microbes per gram.

**Knallgas bacteria**

They have the enzyme hydrogenase which catalizes H2 + ½ O2 🡪 H2O (deltaG = -237 kJ/mol). Ex.: paracoccus, ralstonia eutropha. Such bacteria regenerate NADH with a cytoplasmic hydrogenase that catalizes NAD+ + 2H+ + CO2 + ATP 🡪 NADH + building blocks. In case of absence of the cytoplasmic hydrogenase, it simply makes use of the reverse electron transport chain.

**Nitrifying bacteria**

NH3 and NO2- is oxidized in the process of nitrification. There are two groups: ammonia-oxidizing and nitrite-oxidizing bacteria. Those two work together to produce NO3-. They occur everywhere, where ammonia and oxygen are available like in soil and water. They are also very active in anoxic regions. They generate less energy than other type of bacteria, so their growth is rather slow.

**Oxidation of NH3**

NH3 + O2 + 2H+ 🡪 NH2OH + H2O (with ammonia monooxygenase), NH2OH 🡪 NO2- + 5H+ (with hydroxylamin oxidoreductase). The released electrons from this reaction are transported through cytochrom. ½ O2 + 4H+ 🡪 H2O + 2H+. The protons are pumped across the membrane such that a proton gradient can be built. That proton gradient is used to generate ATP.

**Oxidation of nitrite (NO2-)**

NO2- + H2O 🡪 NO3- + 2H+ (with nitrite oxidoreductase). Electron transport through cytochrom and proton pumping to generate a proton gradient is used to generate ATP.

In agriculture, such bacteria are not wanted, since NH4+ is harder to wash out than NO3- which is easily washed out when raining. They degrade calcite (dt. Kalkstein). They also act as a modern cleaning water station, because they remove nitrogen.

**Phototroph organisms**

There are two types: oxygenic and anoxygenic. Anoxygenic organisms do not make use of oxygen, are historically older and contain either PS I or PS II. E.g. Purpurbacteria. Oxygenic organisms have both photosystems, H2O is the electron donor. E.g. algae and cyanobacteria.

**General form of photosynthesis equation**: CO2 + H2A 🡪 [CH2O] + 2A (+ H2O), A := acceptor.

Phototrophic groups are green non-sulphur bacteria, green sulphur bacteria, cyanobacteria, heliobacteria, purpurbacteria (50% of CO2 fixation is done by bacteria/archaea and the other half by plants).

**Autotroph CO2 fixation**: CO2 + n ATP + 4H+ 🡪 [CH2O] + H2O + n ADP + n P\_i.

**6 types of CO2 fixation pathways**: Calvin cycle, reductive citronic acid cycle, reductive acetyl CoA pathway, 3-hydroxypropionat/malyl CoA cycle, 3-hydroxypropionat/4-hydroxybutyrate cycle, dicarboxylat/4-hydroxybutyrate cycle.

The calvin cycle needs NAD(P)H, ATP, ribulose phosphate, CO2 and RubisCO. One needs 6 CO2 for 1 glucose. General equation: 6 CO2 + 12 NADPH + 18 ATP 🡪 phosphorylated glucose + 12 NADP+ + 18 ADP + 17 P\_i.

Nitrogen fixation is carried out only by some bacteria and archaea. They need the enzyme nitrogenase which cannot work under oxygenic conditions and needs several cofactors such as molybden. Equation: N2 + 8H+ + 8e- 🡪 2 NH3 + H2, using 16-24 ATP. Symbiotic nitrogen fixating bacteria are rhizobia bacteria that live in symbiosis with legumous plants.

**Part 6 – Bacterial natural products**

Bacteria produce a wide variety of **secondary metabolites** which are not indespensable for survival but are needed for defense, pathogenism (adherence), toxins, immune defense damages, communication (quorum sensing via AHL in social bacteria to form biofilms) and in mutualism. Secondary metabolites increase their fitness and growth, but do not influence overall survival. They occur in gene clusters, which is a sequence of genes that produces one or more secondary metabolites. There is usually one gene cluster per bacteria. Bacterial produces are very diverse in function, size and form.

Bacteria can also be modified to produce other secondary metabolites which are useful in medicine or food. Such natural products are anti-bacterial, anti-fungal, anti-inflammatory, anti-viral, immunosuppressive, anti-parasitic agents, insecticides, herbicides.

**Ex.**: Erythromycin is modified to azithromycin which is metabolised more slowly in the body.

**Primary metabolites** such as sugars, amino acids and lipids are indespensable for growth and survival. Deletion results into unviable cells.

**Biosynthetic relationship between primary and secondary metabolites**: primary – link – secondary   
complex carbohydrates – monosaccharides – monosaccharides, complex carbohydrates;  
terpenoid lipids – mevalonic acid/1-deoxyxylulose-5-phosphate – terpenoids;  
proteins – amino acids – peptides, alkaloids;  
fatty acid based lipids – acetyl-CoA/mavonyl-CoA – polyketides;

**Talented natural product producers**

Filamentous actinomycetes (Streptomyces produces rapamycin (immunosuppressivum)).  
Cyanobacteria (Anabeana filamente produces hepatotoxin microcystin-LR).  
Myxobacteria (Sorangium cellulosum produces epothilone D). Also Bascillus spp. or Pseudomona spp.

**Life cycles of two natural product producers**

Streptomyces: free spore 🡪 vegetative mycelium 🡪 air hyphes 🡪 spore production

Myxobacteria: myxospore 🡪 vegetative proliferation 🡪 aggregation 🡪 pile of cells 🡪 fruit body

**Identification of secondary products**

Polyketides: usually contain one long C-chain with -OH groups and other groups.  
Peptides: contain amide bonds (proteinogenic amino acids).  
Alkaloids: contain N-atoms in cycles and few to no amide bonds.  
Sugars: 5 or 6 cyclic with O-atom in cycle (cyclic ether bond). Rings are connected with ether bonds or N-bonds. Also has hydroxy groups.  
Terpenes: contain methyl groups in a 1,5 distance fashion. Can be easily confused with polyketides, therefore, simply count the distance of two methyl groups (must be 5 – also count both methyl groups as first and fifth position).

**Terpenes**

The isopren-rule forms terpenes. Terpenes are made from repeating isopren repeats (with side chain modifications sometimes). There are mono-, sesqui-, di-, tri- and tetraterpenes. Isoprens have pyrophosphate at their tail side (the side farther away from the methyl group) which bind to the either side of another isopren (pyrophosphate is lost in this process). Terpenes are structurally very diverse because one terpensynthase can catalyze several cyclisations leading to different structures.

**Alkaloids and non-proteinogenic peptides**

Alkaloids are made from non-proteinogenic amino acids via the non-ribosomal peptide synthase (=: NRPS) and from normal amino acids. Regular peptides have a short life span in cells, because they are degraded by peptidases and proteases rather fast and can have multiple secondary structure of which some are non-functional. Modified peptides are immune to peptidases and cyclisation bridges (dt. Zyklisierungsbrücken) make for rigid conformational structures (= not multiple open-chain structures).

**How to make a modified peptide**: Firstly, post-translational modifications such as lipidation, acylation, glucosylation etc. may occur. Secondly, the synthesis of peptides from non-proteinogenic building blocks with NRPS.

**NRPS**: it has an anchor site (peptidylcarrier protein domain), adenylation domain (chooses and activates an amino acid and adds it to PCP domain) peptide adding site (condensation domain), an epimerisation site (change L to D configuration of amino acid), thioesterase domain (cuts off peptide and makes cyclisations).

Its products have anti-bacterial or cytotoxic properties, such as lantibiotics and microcins.

**Production of polyketides**

The core polyketide of complex polyketides is made with the type 1 polyketide synthase (PKS). Aromatic polyketides are made with the type 2 PKS which is a sequence of enzymes.

**Mechanism of type 1 PKS**: ACP + acyl transferase 🡪 AT building blocks+ACP + CO2 (CO2 lost), enzyme: ketosynthase. AT building blocks+ACP as growing chain is reduced by ketoreductase, then H2O removed with hydratase, then again reduced via enoyl reductase. (🡪 **collinearity rule**)

**Mechanism of type 2 PKS**: Propionyl-CoA + Malonyl-CoA 🡪 ACP; ACP + malonyl-CoA 🡪 chain elongation (longer ACP) via ketosynthase. Cyclisation enzymes from cyclisations in ACP. Tailoring enzymes the cycles for its final form.

**Parrellels between NRPS and PKS**:

Collinearity rule, module with several enzymes, condensation domain, anchoring domain, adenylation domain (⬄ building block selection domain). Both evolved independent of each other (**convergent evolution**).

**Part 7 – Microbial interactions**

In nature, there is a lot of competition (the fight for nutrients) and cooperation (two or more organisms work together to produce or degrade a metabolite) going on between different species of bacteria. Also, different genes are active during natural conditions, which are not present in laboratory settings such as defense genes and metabolic pathways, since natural conditions are dynamic and not constant like in the lab. They earth itself is a very heterogeneous system with locally, heavily varying physical conditions and places with nearly constant conditions (underwater, desert). There are places that favor oxygenic or anoxygenic species (anoxygenic: underwater at hot springs that release a lot of H2S).

**Def. microbial ecology**: The study of interactions of microbes with other organisms and the environment (for example, exchange of chemicals).

**Def. environmental microbiology**: The study of the diversity and interactions of microbes with their natural environment.

**Interactions of microbes and higher organisms**: Mutualism (both benefit), commensalism (no benefit and no harm for host), pathogenesis/parasitism (harm for host).

Microbial interactions are involved in global chemistry cycles such as oxygen production (cyanobacteria), carbon fixation, nitrogen fixation etc. In evolution, they were indespensable for the creation of eukaryotes and mitochondria. In humans: pathogenesis and symbiosis (gut bacteria).

**Def. population**: All members of a species in a well defined area.  
**Def. natural community**: Associations of populations.  
**Def. habitat**: A subset of the ecosystem which is most suitable for one or few species to live in.  
**Def. ecosystem**: A functional unit of all organisms and their non-living factors (⬄ all biotic and abiotic factors).  
**Def. biodiversity (dt. Artenvielfalt)**: The absolute number of different species.  
**Def. species abundance**: The per centage of a species in a community.

A **niche** is the entirety of all environmetal factors that influence the survival of a species. A **primary niche** is a niche in which a species is most successful. The **micro environment** describes the local environment of a niche that acts on a single organism.

When there are enough microbes in a rather small area, they change their gene expression through quroum sensing (via AHL for exmaple) initiating the formation of a biofilm. A **biofilm** consists of many bacteria in a extracellular polymere substance. They lose their flagellates. For humans, biofilms are responsible for erosion, coverings on ships and colonization in body parts, protheses and contact lenses. A **microbial mat** is a very old biofilm and thus huge biofilm. The oldest existing biofilm dates back 3.5 billions years.

**Def. desulforudis audaxaviator**: An organism that cannot be cultivated in the lab. Such organisms need H2, N2, CO2, SO42- and minerals for survival (auxotrophic organisms – can be confirmed in the analysis of their genomic sequence).

**Habitats**: Pelagic habitats are open water habitats characterized by a low density of nutrients and organisms. Mostly populated by primary producers and oligotrophs. Piezophilic habitats are high pressure and low temperature (2-3°C) habitats. A hydrothermal spring is a place underwater that is warm to hot and releases a lot of anoxygenic electron donors such as H2S.

**The carbon and nitrogen cycle**

**C-cycle**: CH4 and CO2 most imporant molecules. Humus is the biggest source of carbon. Humans increased atmospheric CO2 by 20% in the last 50 years. Chemical reactions:

Sugars 🡪🡪 CO2 (an/aerobic respiration, fermentation), CO2 🡪🡪 sugars (an/oxygenic photosynthesis, acetogenesis), CH4 🡪🡪 CO2 (methanotrophy), CO2 or sugar 🡪🡪 CH4 (methanogenesis).

In syntrophy, a fermenting and a methanogenic organism team up to make use of exergonic substrates by coupling different reactions together. We have:  
**Ethanol fermentation**: 2 acetate + 2 H2O 🡪 4 H2 + 2 acetic acid + 2H+ (endergonic).  
**Methanogenesis**: 4 H2 + CO2 🡪 CH4 + 2 H2O (exergonic).  
Together – interspecies hydrogen transfer: 2 acetate + CO2 🡪 CH4 + acetic acid + 2H+

**N-cycle**: Nitrogen is needed for amino acids and proteins. Chemical reactions:

N2 🡪🡪 NH3 (nitrogen fixation), NH3 🡪🡪 NO2- (nitrification), NO3- 🡪🡪 NH2 (assimilation),  
NO3- 🡪 NO2- 🡪 NO 🡪 N2O 🡪 N2 (denitrification).

Syntrophy in termites consists of a complex microbial interaction of fermenting bacteria, acetogens and methanogens. Protists: glucose 🡪 CO2 + H2 or G6P. Glucose fermenters: glucose 🡪 CO2 + H2. Acetogens: CO2 + H2 🡪 acetate; methanogens: CO2 + H2 🡪 CH4. Termites use acetate for energy.

The analysis of a microbial community can be carried out through growth dependent of indepent analysis.

In growth dependent analysis, one can either change the conditions on the growth medium to select for a species (dt. Anreicherung) or do several smears (dt. Abstriche) on the agar plate to isolate a strain or increase dilution during agar dilution. In **micromanipulation**, one can remove a single cell under a microscope with a capillary and thus inhibit a fast-growing contamination.

In growth independent analysis, one performs (gram) stainings, metagenomic analyses or 16S rRNA analysis.

Their activity in a habitat can be measured through activity measurement of the assignment of specific genes to organisms in metagenomics.

**Def. symbiosis (Anton de Bary)**: An association between unequal organisms which is intimate, specific and lasting.

**Symbiosis**

An organelle has lost most of its metabolic pathways and is obligatory dependent on its host. An endosymbiont is capable of living without symbiosis.

**Endosymbiotic theory (pro-arguments)**: Mitochondria and plastides have their own (small) genome (-> DNA phylogeny), 2 membranes for these organelles, bacterial-like ribosomes, proteins have N-formyl methionine at the terminus.

Evolution of eukaryotes: probably developed from archeas with a cell nucleus. Cell nucleus only developed after the assimilation of endosymbiont. Hydrogenosomes and mitochondria share a similar origin.

There are also obligate endosymbionts with a highly reduced genome that cannot live freely. They occur in insects in bacteriosomes. Those are special organs in cells which are called bacteriozytes and they produce essential amino acids and riboflavin in the case of aphids. The endosymbiont is naturally passed on to the next generation.

**Note**: Hodgkinia cicadicola has the smallest bacterial genome (144kb).

**Ecological role of mutual symbionts**: provide with nutrients, defense, light production (bioluminesence for bait or camouflage underwater), movement in chlorochormatium aggregatum.

Lychen: The association of a fungi and an algea or cyanobacteria or both. Symbiosis completely changes their morphology. Fungi profits from organic material. Both are facultative symbionts.

**Rhizobial infection**

Plant releases flavinoids to signal its presence and to activate/inhibit the transcritption of nod-genes in a rhizobium. Bacteria release nod factor. Root hairs curl in. Bacteria enter the cells and multiply there, forming a thin filament (infection thread) until it reaches the root cells. There, formation of bacteriod stadium occurs. Continuous plant and bacteria cell divison leads to the formation of nodes.

**Def. symbiosomes**: intracellular vacuole-derived plant compartiments.

**Human gut microbiom**: Heterogenous, involved in chemical reactions for energy production, 1-2kg bacterial mass, 10 times more bacteria than human cells, 100 times more genes than human genes, around 500 OTUs per human, composition varies with diet and health state.

Their functions include the production of enzymes for the degradation complex carbohydrates and fermentation to small chain fatty acids. They also produce essential amino acids and vitamin B12 and K amongst others. Another function is development and health: they fight pathogenic microbes, trigger the expression of gut genes in epithelial cells to increase glucose/lipid transport and take up, play a role obesity and play a role in the innate immune system.

**Pathogenesis and infection**

**Process of pathogenesis**: exposure of pathogen, adherance to skin or mucous membrane, invasion through epithelium, colonialisation and growth and production of virulence factors, toxicity and invasion, illness and tissue damage.

**Def. infection**: The entry and facility of an organism into a cell (can be mutualism, commensalism, pathogenesis).

**Def. Virulence**: The number of microbes needed to kill the host. LD50 is the dosis of microbes needed to kill 50% of a host’s cells. Virulence factors allow the pathogen to adhere on the host, damage the host and protect itself and multiply.

Adherence on surfaces during an infection occurs as a mucous layer or capulse, with adhesins, with fimbriae or pili, or with pathogenic receptors that is taken up by the host such that the pathogen can enter the host.

Toxins damage the host and are used to overcome its defense barriers. Endotoxins are released from bacteria while exotoxins adhere to the bacteria.

Clostridium botulinum releases the botulinum toxin which inhibits the release of neurotransmitters in muscles, inhibiting contraction and resulting into botulism.

Clostridium tetani releases the tetanus toxin which binds to inhibitory interneurons inhibiting glycine release. This results into muscular cramps.

**Endotoxins or LPS** are contained in the membrane of most gram-negative bacteria and they are released after their death. They induce high fever, inflammations and in higher concentrations also hemorrhagic shock and death.

**The virulence factors of salmonella**: siderophores (steal Fe3+), type 1 fimbriae for adherence, cytotoxins (no protein synthesis in host and leakage of Ca2+ from cells), Vi capsule antigen, flagellum for movement, H antigen and O antigen (inhibit destruction of phagozytes), anti-phagozytic protein, endotxin in LPS, injectosome, enterotoxin for injection.

Viruelence factors are often encoded in small genomic regions, horizontally acquired, and are known as **pathogenicity islands**.

**Parallels between mutualism and pathogenicity**: similar infection mechanisms, genes horizontally acquired (pathogenicity islands or symbiosis islands), mutualism can result out of pathogenic interactions, commensalism can result into pathogenism if host is weakened.